Note: The Thiabendazole Report of the Cancer Assessment Review Committee was originally finalized on November 16, 1999. Documents which were distributed bearing that date should now be discarded, as the document has been revised to include a consideration of risks to infants and children. This revised version is now the official Final Report and it is dated: February 24, 2000.

HED DOC. NO. 013840

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

THIABENDAZOLE

FINAL REPORT
24-FEBRUARY-2000

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

October 7, 1999

Subject: Thiabendazole-CARC Final Report

From: Sanju Diwan,
Executive Secretary,
Cancer Assessment Review Committee

To: CARC Members

During the May 26, 1999 meeting, the CARC recommended a linear low-dose extrapolation approach for human cancer risk assessment. I would like to bring to your attention that since our last meeting there has been a change in the selection of the quantification approach.

Based on comments received on Thiabendazole-CARC draft report, an Ad Hoc Committee of William Burnam, Mike Ioannou, Sanju Diwan and Patricia Gaunt met to reevaluate the mouse carcinogenicity study. Previously we called the study unacceptable because there were several study deficiencies including variable dosing and protocol, variable sacrifice timing for animals and increased mortality in all dose groups including controls. The CARC, therefore, recommended that a new study be conducted in mice using appropriate dose levels.

After reevaluation of the data, the Ad Hoc Committee concluded that there was a dose-related increase in mortality in both sexes at mid- and high dose at 18 months and at high dose at 15 month. However, the percent survival of animals at 15 and 18 months was adequate to meet the guideline requirement. It was noted that there were sufficient number of animals alive at both time intervals to assess the carcinogenic response (refer to page 8 of the Final Report). However, no treatment-related increase in tumor incidence above background level was observed. Although the dosing was variable, and assuming that the animals received the lowest dose of the range for each dose group, a compound-related effect (i.e. increased mortality) was noted in both sexes at the mid- and high dose at 18 months and at the high dose at 15 months. Therefore, the dosing was considered to be adequate and the study was considered acceptable.

The Ad Hoc Committee reaffirmed that the mode of action data supports the mechanism for thyroid tumor induction and therefore, the MOE approach was selected for the quantification of human cancer risk. This is reflected in the final report.

The CARC members should carefully read the new discussion and the rationale for the point of Departure to be used in MOE calculations.

DATA PRESENTATION: _____
Patricia S. Gaunt, Toxicologist

DOCUMENT PREPARATION: _____
Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam _____

Marion Copley _____

Vicki Dellarco _____

Virginia Dobozy _____

Yiannakis Ioannou _____

Nancy McCarroll _____

Linda Taylor _____

Esther Rinde (comments attached)

Jess Rowland _____

Joycelyn Stewart _____

Clark Swentzel _____

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

Lucas Brennecke, Pathology Consultant _____

Lori Brunsman, Statistical Analysis _____

CONTENTS

EXECUTIVE SUMMARY

On May 26, 1999 the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of thiabendazole. The studies evaluated included a 104-week chronic toxicity/carcinogenicity study in Sprague-Dawley Cr:CD BR rats and a carcinogenicity study in CD-1 mice as well as a mechanistic study submitted by the registrant to support the non-linear mode of action for thyroid tumor induction.

In the chronic toxicity/carcinogenicity study, 50 Sprague-Dawley Cr:CD BR rats/sex/dose received thiabendazole in the diet at 0, 10, 30 or 90 mg/kg/day for 104 weeks. In a carcinogenicity study in mice, thiabendazole was administered in the diet to male and female CD-1 albino mice (50/sex/group) at 0, 37, 92 or 278 mg/kg/day for males and 0, 113, 289 or 770 mg/kg/day for females, respectively for 105 weeks.

The CARC concluded that

- Thiabendazole was carcinogenic to rats because: 1) male rats had significant increases in the pair-wise comparisons of 30 and 90 mg/kg/day dose groups with the controls, for thyroid follicular cell adenomas and combined adenomas/carcinomas; 2) in female rats, there was a statistically significant increase in the pair-wise comparison of the 90 mg/kg/day dose group with the controls, for thyroid follicular cell adenomas. 3) significant increasing trend was observed for thyroid follicular cell adenomas and combined adenomas/carcinomas in both sexes, and 4) the incidences of adenomas in males at 30 mg/kg/day and in both sexes at 90 mg/kg/day exceeded the range for the historical controls. The dosing was considered to be adequate in males and females at 90 mg/kg/day based on a decrease in body weight gain and an increase in liver and thyroid weights accompanied by histopathological changes. The increase in TSH and decrease in T3 levels seen at 90 mg/kg/day were indicative of interference with thyroid-pituitary homeostasis. The CARC considered the thyroid tumors in males (at 30 and 90 mg/kg/day) and females (at 90 mg/kg/day) to be treatment-related. The Committee concluded that, although the mechanistic study did not measure the liver enzyme UDP glucuronosyl transferase, there are sufficient data based on the liver changes and thyroxine clearance to support that the thyroid tumors are likely to be due to increased metabolism of thyroid hormone in the liver. When considered together, the available information suggests that thiabendazole may interfere with thyroid-pituitary homeostasis.

- Thiabendazole was not carcinogenic to male and female CD-1 mice.

Thiabendazole has been tested in *in vitro* and *in vivo* genotoxicity assays. It shows genotoxic potential as a spindle inhibitor resulting in aneuploidy induction. This finding suggests an interaction with non-DNA targets such as tubulin polymerization and/or microtubular formation which is needed for the segregation of chromosomes. The CARC determined that there was no need for additional genetic toxicology studies on thiabendazole.

Structurally-related compounds, including Benomyl and MBC (methyl-2-benzimidazole-carbamate), cause hepatocellular tumors; however, neither cause thyroid effects. Thiophanate-methyl, which is converted to the benzimidazole group structure, is a known inducer of liver tumors in mice and thyroid tumors in rats.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified thiabendazole as **"likely to be carcinogenic to humans "** by the oral route and recommended an MOE approach for the quantification of human cancer risk. This extrapolation is supported by the weight-of-the evidence which suggests that thiabendazole may interfere with thyroid-pituitary homeostasis. Children are not expected to be more susceptible than adults to thiabendazole-induced thyroid cancer.

I. INTRODUCTION

On May 26, 1999, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of thiabendazole.

Dr. Patricia Gaunt of the Reregistration Branch 4 described the 104-week chronic toxicity/carcinogenicity study in Sprague-Dawley Crl:CD BR rats and a life-time carcinogenicity study in CD-1 mice by detailing the experimental design; reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of dose levels tested; and presenting the weight-of-the-evidence for the carcinogenicity of thiabendazole-methyl. Dr. Gaunt also discussed the findings of the mechanistic study submitted by the registrant in support of the hormonal mechanism of thyroid tumor induction.

II. BACKGROUND INFORMATION

Thiabendazole is a systemic fungicide used to control fruit and vegetable diseases such as mold, rot, blight, and stain. It is also active against storage diseases and Dutch Elm disease. In livestock, thiabendazole is also applied to treat roundworms. The PC Code No. is 060101. and the CAS No. is 148-79-8. Its chemical name is 2-thiazol-4-yl) benzimidazole. The chemical structure is shown below, Figure 1.

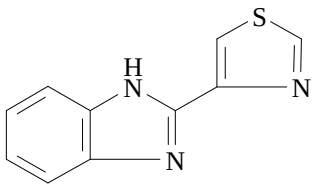


Figure 1**.** *Thiabendazole*

III. EVALUATION OF CARCINOGENICITY EVIDENCE

1. Rat 106-Week Dietary Chronic Toxicity/Carcinogenicity Study

Reference: Squibb R.E. (1993). Thiabendazole: 106-Week Dietary Toxicity/Carcinogenicity Study in rats. Study conducted by Hazleton Washington, Inc. Study Identification Number 618-67/TT #90-9009. MRID # 43593201. Unpublished study.

a. Experimental Design

Groups of 50 Sprague-Dawley Crl:CD BR rats/sex/dose received thiabendazole (>98.9%) at dosages of 0, 10, 30, or 90 mg/kg/day (achieved average doses of 0, 10.1, 30.2, or 91.8 mg/kg/day) for 104 weeks. All sacrifices were made at 106 weeks.

b. Discussion of Tumor Data and Comparison With Historical Control Data

Male rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 90 mg/kg/day dose group with the controls, for thyroid follicular cell adenomas and combined adenomas/carcinomas, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 30 mg/kg/day dose group with the controls (Table 1a) at $p < 0.01$ for thyroid follicular cell adenomas and at $p<0.05$ for thyroid follicular cell combined adenomas/carcinomas. The incidences for the mid- and high-dose males and high-dose females exceeded the range for the historical controls (Table 2).

Female rats had significant increasing trends in thyroid follicular cell adenomas and combined adenomas/carcinomas, both at $p < 0.05$. There was a significant difference in the pair-wise comparison of the 90 mg/kg/day dose group with the controls for thyroid follicular cell adenomas at $p < 0.05$ (Table 1b).

No statistically significant trend in the incidence of any other neoplasm in either sex was observed.

Table 1a. Thiabendazole - Sprague-Dawley Crl:CD BR Rat Study

Male Thyroid Follicular Cell Tumor Rates[+] and
Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (mg/kg/day)			
	0	10	30	90
Adenomas	0/97	1/46	5[a]/47	5/46
(%)	(0)	(2)	(11)	(11)
p =	0.003**	0.322	0.003**	0.003**
Carcinomas	1[b]/97	0/46	0/47	1/46
(%)	(1)	(0)	(0)	(2)
p =	0.353	0.678[n]	0.674[n]	0.541
Combined	1/97	1/46	5/47	6/46
(%)	(1)	(2)	(11)	(13)
p =	0.003**	0.541	0.014*	0.005**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First adenoma not in an accidental kill animal observed at week 92, dose 30 mg/kg/day.
[b]First carcinoma observed at week 105, dose 0 mg/kg/day.
[n]Negative change from control.

Note: Accidental kill animals are not included in this analysis. One accidental kill animal in the 90 mg/kg/day dose group had an adenoma.

Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 1b. Thiabendazole - Sprague-Dawley Crl:CD BR Rat Study

Female Thyroid Follicular Cell Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)			
	0	10	30	90
Adenomas	3[a]/82	0/36	1/43	5/44
(%)	(4)	(0)	(2)	(11)
p =	0.013*	-	-	0.047*
Carcinomas	1[b]/38	0/16	0/22	0/24
(%)	(3)	(0)	(0)	(0)
Combined	4/82	0/36	1/43	5/44
(%)	(5)	(0)	(2)	(11)
p =	0.032*	-	-	0.096

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at week 81, dose 0 mg/kg/day.
[b]First carcinoma observed at week 105, dose 0 mg/kg/day.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Historical control data from Sprague-Dawley rats from 20 studies conducted by Covance/Hazleton Laboratories from 1988-1998.

Males			
Tumor Type	Total No. Rats	Mean No. (%)	Range (%)
Thyroid			
Follicular cell adenoma	1020	31 (3.1%)	0-8.3%
Follicular cell carcinoma	1020	19 (1.9%)	0-8%
Females			
Thyroid			
Follicular cell adenoma	973	10 (1.1%)	0-4%
Follicular cell carcinoma	973	8 (0.9%)	0-2%

c. Non-neoplastic Lesions and Other Findings:

The statistical evaluation of mortality (Brunsman, 1999) indicated increasing mortality with increasing doses of thiabendazole in female rats. Male rats showed no significant incremental changes in mortality with increasing doses of thiabendazole. Thiabendazole had no significant effect on clinical signs, food consumption, ophthalmoscopic findings, urinalysis, or gross pathology. Systemic signs of toxicity in the treated groups were as follows: 1) body weight gains were generally lower (↓7%-30%) throughout the study for the mid- and high-dose males and high-dose females. Reduced body weight gains (↓15%, 28%, and 19%, $p \leq 0.05$) for the mid- and high-dose males and high-dose females, respectively, compared to the controls were observed at week 103. A reduced body weight gain (↓10%, not statistically significant) was also noted at this time for the mid-dose females.

Significant increases (↑36%-79%, $p \leq 0.05$) in total serum cholesterol observed in the high-dose group were judged to be treatment-related. The incidence of selected non-neoplastic lesions is shown in Table 3. In the high-dose males, increased (↑29%, $p \leq 0.05$) relative (to body) liver weights and thyroid diffuse follicular cell hypertrophy (4/50 treated vs 0/50 controls) were observed in the high-dose males. An increased incidence of centrilobular hepatocellular hypertrophy was seen in mid and high-dose males (7/50 and 28/50, respectively vs 0/50 controls). In high-dose females, increased relative thyroid weights (↑45%, $p \leq 0.05$), increased incidences of thyroid focal cystic follicular cell hyperplasia (6/50 treated vs 2/50 controls) and diffuse follicular cell hypertrophy (2/50 treated vs 0/50 controls) were observed.

Table 3: Incidence of selected non-neoplastic lesions in rats dosed with thiabendazole for 104 weeks[a]

Observation	Dose Group (mg/kg/day)				
	Control 1	Control 2	10	30	90
Males					
Thyroid					
Diffuse follicular cell hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	1/50 (2)	4/50 (8)
Liver					
Centrilobular hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	7/50 (14)	28/50 (56)
Females					
Thyroid					
Diffuse follicular cell hypertrophy	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	2/50 (4)
Focal cystic follicular cell hyperplasia	2/50 (4)	1/50 (2)	0/50 (0)	3/50 (6)	6/50 (12)

a Data were extracted from study report, page 2127

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

In light of the systemic effects observed, dosing was considered to be adequate for assessing the carcinogenic potential of thiabendazole in the rat. The body weight gain was decreased throughout the study for the mid- (decreased 17% and 13% over control groups 1 and 2, respectively), high-dose males (decreased 30% and 27% over controls 1 and 2, respectively), and high-dose females (decreased 19% and 19% over control 1 and 2, respectively). In a 13-week mechanistic study (MRID 43593202), at the end of dosing period, the mean body weights of 90

and 270 mg/kg/day dose males were 12% and 32% lower than controls, respectively. In the rat subchronic study (MRID 42942802), total mean body weight gains at 13 weeks were decreased in 40 mg/kg/day males (17%) and 160 and 320 mg/kg/day males (41 and 69%, respectively) and females (38 and 57%, respectively). In the present study, the following evidence of liver pathology was observed: 1) increased relative liver weights in high-dose males (↑29%), 2) an increased incidence of centrilobular hepatocellular hypertrophy in mid- and high-dose males (7/50 and 28/50, respectively vs. 0/50 controls). The following evidence of thyroid pathology was observed: 1) increased relative thyroid weights in high-dose females (↑45%); 2) thyroid diffuse follicular cell hypertrophy observed in the high-dose males (4/50 treated vs. 0/50 controls) and high dose females (2/50 treated vs. 0/50 controls); 3) increased incidences of thyroid focal cystic follicular cell hyperplasia in high-dose females (6/50 treated vs. 2/50 controls). The CARC concluded that the thyroid tumors in mid- and high-dose males and high-dose females were treatment-related because the incidences exceeded the range for the historical controls, there was progression from non-neoplastic changes and dose-response was evident in males.

2. Mouse Life-time Carcinogenicity Study

Reference: Bagdon W, Bokeman B , and Zwickey R (1980) Thiabendazole: Lifetime Carcinogenic Study in Mice. Study conducted by Merck Institute for Therapeutic Research (West Point, Pennsylvania), Laboratory report number TT #77-014-0, MRID 00031447. Unpublished study.

a. Experimental Design

In a carcinogenicity study, Thiabendazole (98.9%) was administered to 50 mice (Charles River CD-1)/sex /dose in diet at dose levels of 0, 36, 92 or 278 mg/kg/day for males and 0, 113, 289 or 770 mg/kg/day for females, respectively; dose range: 0, 37, 92 and 278 (0, 31-42, 63-121, 184-372 mg/kg/day, respectively) for males and 0, 113, 289 and 770 (0, 94-131, 209-368, and 534-1005 mg/kg/day, respectively) for females for 105 weeks. All sacrifices were made at 105 weeks.

b. Discussion of Tumor Data

There were no treatment-related neoplastic lesions detected in the animals when compared to controls.

c. Non-neoplastic Lesions and Other Findings:

There was a dose-related increase in mortality in both sexes at the mid- and high dose at 18 months and at the high dose at 15 months. However, the percent survival of animals at 15 and 18 months was adequate to meet the guideline requirement and is given below for the Control 1, Control 2, Control 3, LDT, MDT, and HDT groups respectively:

For females at 15 months: 100%, 96%, 96%, 98%, 94%, and 50%
For males at 15 months: 86%, 98%, 98%, 96%, 74%, and 70%
For females at 18 months: 92%, 88%, 80%, 90%, 66%, and 26%
For males at 18 months: 78%, 72%, 70%, 72%, 56%, and 40%.

Body weight gains were significantly lower in high dose females (28%) and males (18%). There was an increase in the absolute liver weight of high-dose females, and an increase in the relative liver weight in mid-dose females and high-dose males and females. There was an increase in the relative liver: brain weight ratio in high-dose males and females.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

It was noted that there were sufficient number of animals alive at both time intervals to assess the carcinogenic response. However, no treatment-related increase in tumor incidence above background level was observed. Although the dosing was variable, and assuming that the animals received the lowest dose of the range for each dose group, a compound-related effect (i.e. increased mortality) was noted in both sexes at the mid- and high dose at 18 month**s** and at the high dose at 15 month**s**. Therefore, the dosing was considered to be adequate and the study was acceptable.

IV. Toxicology Data

1. Metabolism

In a rat metabolism study (MRID 42114701), [phenyl-U-^{14}C] thiabendazole (99.1% a.i.) was administered to five Crl:CD BR strain rats/sex/dose by gavage as a single dose at 25 or 400 mg/kg or as a single dose at 25 mg/kg following a 14-day pretreatment with unlabeled thiabendazole at 25 mg/kg/day.

[^{14}C]Thiabendazole was readily absorbed by male and female rats following oral dosing. The rate of urinary excretion for both sexes in the high dose groups was lower during the initial 24 hours compared to the low dose groups. For all test groups, ~51-73% of the dose was excreted in the urine during the first 48 hours. Dose rate and pretreatment with thiabendazole had no apparent effect on absorption. Within 168 hours of dosing at 25 mg/kg (with or without pretreatment) or 400 mg/kg, 94.3-98.9% of the administered dose was recovered from both sexes, of which 67.3-74.6% was in the urine, 21.3-26.7% was in the feces, and 0.3-2.5% was in the cage washes. Based upon data from a preliminary study, significant levels of radioactivity were not expected in organic volatiles. For all dose groups, concentrations of radioactivity were highest in the cellular fraction of the blood. Residue levels in tissues/organs were generally highest in heart, lungs, spleen, kidneys, and liver, and lowest in fat.

The metabolic profile in urine was similar between the test groups; no unchanged thiabendazole was detected in urine. The majority of the administered dose was recovered in the urine identified as the glucuronide conjugate of 5-hydroxythiabendazole (7.3-21.3% of dose) and the sulfate conjugate of 5-hydroxy thiabendazole (23.7-44.9% of dose). Males produced lower amounts of the glucuronide conjugate and higher amounts of the sulfate conjugate than females. Minor amounts ($\leq$1% of dose) of free 5-hydroxythiabendazole (HTBZ) were present in urine from rats from all dose groups. HPLC analyses of fecal extracts isolated minor amounts of thiabendazole from males in the preconditioned low dose group and from both sexes treated in the high dose groups, and minor amounts of free HTBZ in all dose groups.

The data indicate that renal excretion is the primary pathway for the elimination of thiabendazole from rats. At the low dose level, it was shown that thiabendazole oxidizes to form 5-hydroxythiabendazole, followed by conjugation to form glucuronide and sulfate conjugates of 5-hydroxythiabendazole.

2. Mutagenicity

The acceptable genetic toxicology studies on thiabendazole indicate that the compound is not mutagenic in bacteria or clastogenic *in vitro* in mammalian cells in studies conducted without S9 activation. Thiabendazole did not cause DNA strand breaks in cultured rat hepatocytes and was negative for structural chromosome aberrations in rats. In contrast to the negative findings from the acceptable required submitted studies, thiabendazole has been shown by several investigators in the published literature to induce micronuclei in mouse bone marrow with $\geq$75% of the scored micronuclei staining positive for kinetochore (KC+). The finding of increased KC+ micronuclei suggests an aneuploidy effect (adverse effects on chromosome numbers) since micronuclei staining positive for kinetochore are presumed to contain intact chromosomes while those staining negative for kinetochore, contain chromosome fragments resulting from structural chromosome damage (i.e., a clastogenic effect). There is also evidence from the literature that thiabendazole induces aneuploidy, not only in somatic cells *in vivo*, but also in germinal cells (secondary spermatocytes and oocytes) of whole animals, at high toxic doses. Thiabendazole has also been shown to inhibit the *in vitro* polymerization of porcine brain tubulins. This finding is in agreement with the proposed mechanism by which benzimidazole-related chemicals induce aneuploidy (i.e., through interference with microtubule assembly). Similarly, the positive results from the *in vivo* micronucleus assays are consistent with the data from other benzimidazole analogs (e.g., the common metabolite of benomyl and thiophanate-methyl, methyl-2-benzimidazole carbamate (MBC), and benomyl) indicating that these compounds are confirmed inducers of aneuploidy. The above compounds also have the mouse liver as the target organ for carcinogenesis. Benomyl, MBC, thiophanate-methyl and thiabendazole contain the benzimidazole moiety and are positive for aneuploidy induction.

Neither a mammalian cell gene mutation assay nor an *in vitro* chromosome aberration assay in the

presence of S9 activation were conducted. Hence, the acceptable studies do not satisfy the current mutagenicity guideline requirements. It was concluded, however, that since there is confirming evidence that thiabendazole is aneugenic, no further genetic toxicology testing is required. Summaries of the acceptable mutagenicity studies are presented below; and studies from the open literature, summarized by Dr. Irving Mauer (1999), are also discussed.

GENE MUTATIONS

1) *Salmonella. typhimurium*/*Escherichia coli* mammalian microsome gene mutation assay: The test was negative up to cytotoxic and insoluble levels ($\geq$300 μg/plate) with or without S9 activation. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a bacterial gene mutation assay (MRID 42361801).

2) Host mediated assay: The test was negative up to the highest dose tested (1000 mg/kg via oral gavage once daily for 5 consecutive days) using *S. typhimurium* G46 as the indicator organism and ICR male mice as the host. The study is classified as Acceptable nonguideline (MRID 00098002).

CHROMOSOME ABERRATIONS

3) *In vitro* mammalian cell cytogenetic assay in human embryo fibroblasts: The test is negative up to the highest dose tested (50 μg/mL) without S9 activation. The study is classified as Acceptable but does not satisfy the requirements for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic assay (MRID 00098002).

4) *In vitro* mammalian cell cytogenetic assay in WI-38 human fibroblasts: The test is negative up to precipitating levels (1000 μg/mL) without S9 activation. The study is classified as Acceptable but does not satisfy the requirements for FIFRA Test Guideline 84-2 for *in vitro* cytogenetic assay (MRID 00125297).

5) *In vivo* cytogenetic assay: The test was negative in Wistar rats administered single doses of 10-1000 mg/kg by oral gavage or 30-300 mg/kg once daily for 5 consecutive days. Lethality was seen in the high-dose group but there was no evidence of bone marrow cytotoxicity. The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for an *in vivo* cytogenetic assay (MRID 00098002).

6) *In vivo* bone marrow chromosome aberration assay: There were no significant increases in the incidence of chromosome aberrations at any sampling time in male CRL:CD-1 mice given a single oral dose of thiabendazole (99.8% purity) in methylcellulose at levels of 200, 667, and 2,000 mg/kg bodyweight. The positive control induced significant increases in cells with chromosome aberrations. The study is classified as Acceptable and satisfies the guideline (84-2) requirements for an *in vivo* cytogenetic assay (MRID 43328304).

OTHER GENETIC MECHANISMS

7) *In vitro* alkaline elution assay in primary rat hepatocytes: The test is negative up to a precipitating level (1.3 mM). The study is classified as Acceptable Nonguideline (MRID 41170103).

OTHER INFORMATION

Literature studies have been summarized by Dr. Irving Mauer (Mauer, I., 1999). This review indicated that thiabendazole is not mutagenic or clastogenic but does possess weak, although definitive, aneugenic activity in both somatic and germinal cells. A brief summary of the open literature studies supporting these conclusions follows:

As part of the European Communities co-ordinated effort to validate aneuploidy detecting test systems, Van Hummelen et al., (1995) found that thiabendazole was negative in the *in vitro* cytochalasin-B micronucleus test on human lymphocytes up to insoluble and cytotoxic concentrations (300 μM +/- S9). Similar negative results were obtained by Migliore and Nieri (1991) using this test system up to cytotoxic doses ($\geq$200 μg/mL) of thiabendazole. In contrast, Sbrana et al. (1993) found thiabendazole to be positive for c-mitotic effects and increased mitotic indices in human lymphocytes at 100 to 900 μg/mL. The peak effect was seen at concentrations near the cytotoxicity limit (900 μg/mL).

Thiabendazole, which is a member of the benzimidazole class of compounds, has been reported to inhibit the *in vitro* polymerization of porcine tubulin (Brunner et al., 1991). This finding for thiabendazole is consistent with the known mechanism of aneuploidy induction by the benzimidazole class of compounds (i.e., *in vitro* inhibition of yeast and mammalian tubulin assembly with impairment of the spindle apparatus and resulting aneuploidy in the daughter cells) (Albertini, 1991). Other representative members of the benzimidazole class include the common metabolite of benomyl and thiophanate-methyl, methyl-2-benzimidazole carbamate (MBC) and benomyl.

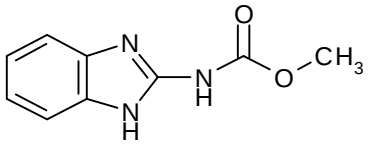
In vivo, thiabendazole induced significant and dose-related increases in micronuclei in bone marrow cells harvested from CFW mice receiving 50, 100 or 200 mg/kg by intraperitoneal injection (ip) (Mudry de Pargment et al., 1987). The same investigators also observed significant sister chromatid exchanges *in vivo* at 200 mg/kg and a significant increase in abnormal anaphase-telophase figures in cultured Chinese Hamster ovary (CHO) cells at 0.6 and 0.24 μg/mL. Marrazzini et al. (1994) also noted significant increases in micronuclei at 250 and 500 mg/kg thiabendazole but no significant effects on chromosome structure. In agreement with these data, Gudi et al. (1992) showed significant and dose-dependent increases in the total micronucleated polychromatic erythrocytes (MPCEs) frequency harvested from the bone marrow of CD-1 mice administered ip injections of 400, 800 or 1200 mg/kg thiabendazole. Of the induced MPCEs, 83,

75 and 79%, respectively, contained kinetochore. The kinetochore response was dose related and significant. Kinetochore containing micronuclei are considered to result from whole chromosomes or centric fragments and are indicative of aneuploidy while micronuclei arising from clastogens do not contain kinetochore. Additional confirmation of micronuclei induction by thiabendazole in rodent bone marrow comes from the work of Leopardi et al. (1993) showing a slight but significant increase in MPCEs in the bone marrow of (C57BL/Cne x C3H/Cne) F_1 male mice at the only dose tested (62.5 mg/kg via ip injection). The same investigators evaluated hyperploidy in the germ cells of the above mouse strain at 62.5-250mg/kg and found a significant increase in the hyperploidy index of secondary spermatocytes sampled 18 hours after treatment with the highest dose tested of thiabendazole (250 mg/kg). On the other hand, Miller and Adler (1992) found no evidence of hyperploidy in the secondary spermatocytes of (102/E1xC3H/E1)F_1 mice harvested 6 or 22 hours postdosing. Although the data of Leopardi et al. are in conflict with the data of Miller and Alder, it is noteworthy that both groups of investigators used different sampling times. Since sampling times are critical for spermatocytes, it is possible that different stages of maturation with different sensitivities to the test material were evaluated. Further support of the aneuploidogenic activity of thiabendazole for germinal cells was furnished by Mailhes et al. (1997). In this study, superovulating ICR female mice were administered 50-150 mg/kg thiabendazole via ip injection; ovulated oocytes were collected 17 hours after compound administration and processed for cytogenetic analysis. Results show that the ratio of metaphase I to metaphase II oocytes and the percentage of polyploid oocytes were unaffected by treatment; however, a small but statistically significant increase in the frequency of hyperploidy was reported at 100 mg/kg. Although the response was not dose-related, the investigators stated that thiabendazole at all doses tested reduced both the ovulation rate as well as the number of oocytes collected per ovulatory female; these effects were significant at 50, 150 or 200 mg/kg but not at the dose causing an increased frequency of hyperploidy (100 mg/kg).

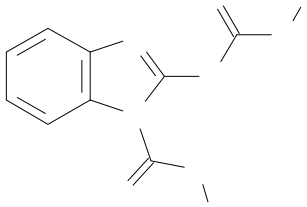
3. Structure-Activity Correlations

Benomyl, thiabendazole, and MBC contain the benzimidazole moiety and have similar carcinogenicity target organs suggesting a *possible* common mechanism. Benomyl causes hepatocellular adenomas and carcinomas in two genetically related strains of mice (CD-1 and Swiss SPF) and has been classified as a Group C carcinogen (EPA, 1998a). Thiophanate-methyl is not a benzimidazole, but is converted into a benzimidazole group structure when metabolized. It causes hepatocellular adenomas in male and female mice and thyroid adenomas in male and female rats and is classified as “likely to be carcinogenic to humans “ (CARC 1999). However, thiabendazole has not been shown to cause liver tumors in mice. **However, there is no mechanistic information available to assess the contributory role of the benzimidazole moiety toward carcinogenic activity. Furthermore, it should be pointed out that benomyl and MBC are carbamates which may contribute to carcinogenicity through other mechanisms.**

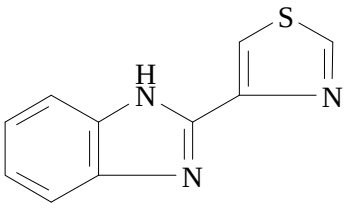
MBC



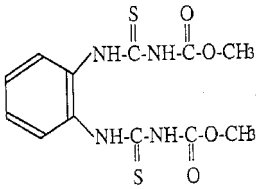
BENOMYL



THIABENDAZOLE



THIOPHANATE-METHYL



Subchronic/Chronic Toxicity Studies

I. Fourteen Week Oral Toxicity (Gavage) Study in Rats

In an oral toxicity (gavage) study in rats (MRID 42942801), thiabendazole (98.9%) was administered to Crl:CD (SD) albino rats (20/sex/dose) by gavage at dosages of 0, 25, 100, or 400 mg/kg/day for 14 weeks. Very slight to slight centrilobular hypertrophy was observed in the livers of 13/20 males and 11/20 females, and absolute and relative liver weights were increased 42-77%. Slight to moderate follicular cell hyperplasia was observed in the thyroids of 18-19/20 rats per sex; absolute and relative thyroid weights were increased 56-64% and 104-136%, respectively. Centrilobular hypertrophy was observed in the livers of 2/20 males and 5/20 females; follicular cell hyperplasia was observed in the thyroids of 8/20 males and 8/20 females. Absolute

liver weights (females only) and relative liver, kidney, and thyroid weights were increased 10-29%. The LOAEL for this study is 100 mg/kg/day, based on histopathological changes of the liver and thyroid. The NOAEL is 25 mg/kg/day. The study was acceptable/guideline.

ii. Fourteen Week Oral Toxicity (Feeding) Study in Rats

In a 14 week oral toxicity (feeding) study in rats (MRID 42942802), thiabendazole (99.4% a.i.) was administered to Crl:CD(SD) albino rats (10/sex/dose) in the diet at nominal dose levels of 10, 40, 160, or 320 mg/kg/day (achieved doses: 1, 9.4, 37, 149, and 302 mg/kg/day for males; 0, 9.4, 38, 152, and 302 mg/kg/day for females) for 13 weeks.

The total mean body weight gains at 13 weeks were decreased in 40 mg/kg/day males (17%) and 160 and 320 mg/kg/day males (41 and 69%, respectively) and females (38 and 57%, respectively). There were statistically significant increases in liver and thyroid weights with increasing dosages. Statistically significant increases in absolute liver weights were observed at 160 mg/kg/day and higher in females and relative liver weights at 40 mg/kg/day in females and 160 mg/kg/day and higher in males and females. Statistically significant increases in absolute thyroid weights were observed in females dosed at 160 mg/kg/day and higher and relative thyroid weights in males and female rats dosed at 160 mg/kg/day and higher.

Histopathological examination revealed treatment-related changes in the liver and thyroid. At 40, 160, and 320 mg/kg/day, centrilobular hypertrophy was observed in the livers of females(7/10, 9/10, and 9/10, respectively) and females (1/10, 9/10, and 9/10, respectively). At these same doses, follicular cell hypertrophy was observed in the thyroids of males (1/10, 2/10, and 6/10, respectively) and females (3/10, 10/10, and 10/10, respectively). The LOAEL for this study is 40 mg/kg/day (37 mg/kg/day), based on reduced body weight gains in males (17%) and histopathological changes in the bone marrow, liver, and thyroid. The NOAEL is 10 mg/kg/day (9.4 mg/kg/day). The study was acceptable/guideline.

iii. Chronic Toxicity Study in Dogs

In a chronic toxicity study (MRID 42809701), thiabendazole (99% a.i.) was administered orally in capsules to four beagle dogs/sex/dose at dose levels of 0, 10, 40 or 160 mg/kg/day for 52 weeks.

Dogs lost weight during the first half of the study primarily due to emesis. One mid-dose male dog died of acute hepatitis after two weeks of treatment.

Clinical pathology revealed treatment-related changes in some of the hematology parameters; clinical chemistry and urinalysis parameters were unaffected by treatment. Both sexes were mildly anemic, with decreased red blood cell counts, hematocrit, and hemoglobin values, and had increased activated partial thromboplastin time (10-14%) and platelet counts (51-65%).

However, none of the values were outside of the historical control range. There was also a higher incidence of polychromasia and hypochromia compared to the controls during weeks 4, 12, and 26.

At terminal sacrifice, treatment-related changes in organ weights and incidence of histopathological findings were observed. The absolute and relative (% of body weight) liver weights were statistically significantly ($p \leq 0.05$) higher in mid- (14 and 20%, respectively, combined sexes) and high- (37 and 41%, respectively, combined sexes) dose animals. In high-dose animals, absolute thyroid weights were increased by 22% (not significant), while relative thyroid weights were increased by 33% ($p \leq 0.05$).

Histopathological evaluations identified lesions in the liver, thyroid, gallbladder, kidney, urinary bladder and spleen. Livers exhibited slight to moderate bile duct vacuolation in mid- (4/4 males; 2/4 females) and high- (3/4 males; 3/4 females) dose animals. Thyroids had very slight follicular enlargement in high-dose females (1/4), while very slight to slight follicular cell hypertrophy was observed in high-dose males (1/4) and females (2/4). Dogs in the 10, 40 and 160 mg/kg/day treatment groups had gallbladders which exhibited cytoplasmic lipid vacuolation and discolored foci of the mucosa; dose-related increases in severity (very slight to marked) were observed. However, kidneys of mid- (3/4) and high- (4/4) dose females showed very slight to slight distal tubule vacuolation, compared to 1/4 females each in the control and low-dose groups. Urinary bladders of all high-dose dogs had very slight to slight epithelial cytoplasmic inclusions; this finding was also observed in 3/4 males and 2/4 females in the mid-dose group. However, the toxicological significance of the above findings could not be determined. Spleens exhibited very slight to slight increases in erythropoiesis in mid- (1/4 males; 1/4 females) and high- (2/4 males; 3/4 females) dose animals; hemosiderin deposits were observed in mid- (2/4 males; 2/4 females) and high- (1/4 males; 4/4 females) dose animals. The LOAEL is 40 mg/kg/day, based on increased liver weight, as well as splenic erythropoiesis and hemosiderosis in both sexes. The NOAEL is 10 mg/kg/day. The study was acceptable/guideline.

5. Mechanistic Study

In a thyroxine clearance study (MRID 43593202), thiabendazole (99.8% a.i.) was administered to 35 Crl:CD(SD) BR male rats/dose in the diet at nominal dose levels of 0, 10, 90, or 270 mg/kg/day (actual 0, 10.15, 91.14, and 235.23 mg/kg/day). After 13 weeks of treatment, 15 rats/dose were sacrificed for pathological evaluation (liver and thyroid only). During week 14 of the study, blood samples from 5 rats/dose were used for evaluation of homeostasis of thyroid hormones and thyroid stimulating hormone; animals were discarded after the final blood sampling. All remaining rats (14 to 15/dose) were sacrificed after 13 weeks of the treatment-free recovery phase and evaluated pathologically (liver and thyroid only).

Mortality and clinical signs of treated animals were unaffected by treatment. After 13 weeks of dosing, mean body weights of the mid- and high- dose animals were 12% and 32% lower than controls, respectively. At the end of the recovery phase, their mean body weights for each of these dose groups were 13% lower than the controls. Pathological evaluations of the thyroid and liver showed increased absolute and relative organ weights and increased incidence of microscopic lesions. In the mid- and high-dose animals, absolute thyroid weights were increased by 20 and 40%, respectively, and relative (to body) thyroid weights, by 26% and 103%, respectively. Relative liver weights were also increased by 7% and 25%, in mid- and high-dose animals, respectively (Table 4). Histopathological examination revealed dark foci in the thyroids of the 6/15 high-dose animals and very slight to slight diffuse follicular cell hyperplasia in the thyroid of 10/15 mid- and 12/16 high-dose animals. Very slight to slight hepatocellular centrilobular hypertrophy was detected in all (15/15) mid-dose animals and 15/16 high-dose animals. The thyroid and liver lesions were not observed in any of the low-dose and control main study animals or in any of the recovery phase animals.

Table 4: Absolute and relative (to body) organ weights (g) in 15 male rats/dose treated with thiabendazole for 13 weeks.

Organ Weight		Dose (mg/kg/day)			
		0	10	90	270
Thyroid	Absolute	0.02	0.02	0.024	0.028
	Relative	0.0038	0.0040	0.0048	0.0077
Liver	Absolute	15.36	14.78	15.42	13.15
	Relative	2.88	2.89	3.09	3.59

[a] These data were extracted from study report Table B-2, page 97.

Thyroid homeostasis (serum T_3, T_4 and TSH) was evaluated during treatment weeks 2, 4, 8, and 13 and weeks 6 and 13 of the recovery phase. T_3 levels decreased by 5-10% in mid-dose and 11-19% in high-dose animals compared to controls; TSH levels were increased by 66-160% in mid-dose and 65-189% in high-dose animals (Table 5). Evaluations carried out during week 14 of the study, showed effects in mid- and high-dose animals. For high-dose animals, statistically significant increases in thyroxine clearance (44%, $p=0.001$), volume of distribution (V_d, 64%,$p<0.001$), rate of elimination (k_{el}, 13%, $p<0.001$), and half-life ($T_{1/2}$, 16%, $p<0.001$). At the mid-dose significant increases in k_{el} (18%, $p<0.001$), $T_{1/2}$ (23%, $p<0.001$) and in V_d (22%, $p=0.004$) were observed, while no differences in thyroxine clearance was observed (Table 6). At the end of the recovery period, TSH levels in the mid- and high-dose animals were comparable to control values while T_3 serum levels were higher (9%-19%). T_4 levels were comparable to controls throughout both the treatment and recovery periods.

Table 5. Mean T_3 and TSH [a]

Study Group	Week	Nominal Dose (mg/kg/day)			
		0	10	90	270
T_3 (ng/dL)					
Main Study	Pretest	101	106	108	102
	2	97	97	87	79
	4	109	111	102	93
	8	81	85	77	66
	13	104	106	97	92
Recovery Phase	Pretest	122	120	122	122
	19	90	103	100	106
	26	92	96	100	109
TSH (µU/mL)					
Main Study	Pretest	50	64	77	71
	2	46	56	88	75
	4	28	37	72	80
	8	96	91	159	190
	13	73	65	128	144
Recovery Phase	Pretest	50	59	63	62
	19	75	72	61	59
	26	88	98	86	86

[a]These data were extracted from study report Tables A-9 and A-11, pages 78 through 81 and 86 through 89, respectively.

Table 6. Thyroxine elimination rate constant (k_{el}), half-life ($T_{1/2}$), volume of distribution (V_d), and systemic clearance (Cl_s) determined in 5 male rats/dose treated with thiabendazole for 13 weeks.[a]

Variable	Nominal Dose (mg/kg/day)			
	0	10	90	270
K_{el}	-0.0374 ± 0.0008 [b]	-0.0393 ± 0.0025	-0.0306 ± 0.0008 ‡‡	-0.0325 ± 0.0012 ‡‡
$T_{1/2}$	18.50 ± 0.39	17.66 ± 1.12	22.67 ± 0.61 ‡‡	21.36 ± 0.78 ‡‡
V_d [c]	15.65 ± 0.45	16.63 ± 0.33	19.07 ± 0.57 ‡	25.52 ± 1.72 ‡‡
Cl_s [c]	0.59 ± 0.01	0.65 ± 0.04	0.58 ± 0.02	0.83 ± 0.08 ‡‡

[a] These data were extracted from study report Table 2, page 175.
[b] Values are geometric mean ± S.E. (n = 5)
[c] Normalized for 100 g body weight.
‡, ‡‡ Statistically significant increasing trend at p=0.004 and p≤0.001, respectively.

These data support the hypothesis that thiabendazole alters thyroid hormone homeostasis in male rats resulting in hypothyroidism. The study authors contend that the primary effect of thiabendazole is on the liver, resulting in hepatocellular hypertrophy [microsomal enzyme induction presumed, but not measured in the study]. Enhanced hepatic metabolism of the thyroid hormones leads to decreased serum levels. The decreased serum levels of the hormones causes an increased release of TSH. The higher serum TSH levels, in turn, cause thyroid hypertrophy and hyperplasia. The study was classified as Acceptable/nonguideline.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity:

! The CARC concluded that thiabendazole was carcinogenic in male and female rats because: 1) a treatment-related increased incidence of thyroid follicular cell adenoma was noted in the mid- and high-dose males (5/47, 11% and 5/46, 11%, p<0.01 vs 0/97, 0% in controls) and high-dose females (5/44, 11%, p<0.05 vs 3/82, 4% in controls). There was also increase in the combined incidence of adenomas/carcinomas at 30 and 90 mg/kg/day in males (5/47, 11% and 6/46, 13%, vs 1/97, 1% in controls). These increases were statistically significant by pair-wise comparisons with the controls. Thyroid tumors were considered by the CARC to be treatment-related because of the progressive nature of the tumor and evidence of dose-response. Also, in the male and female rats, there was a statistically significant increasing trend (p<0.01 in males and p<0.05 in females) in the incidence of thyroid follicular cell adenomas and combined adenomas/carcinomas with increasing dose; 2) the incidence of thyroid follicular cell adenomas in the mid- and high-dose males and high-dose females exceeded the range for historical controls (males: 0%-8.3%; females: 0%-4%); and 3) preneoplastic changes such as centrilobular hepatocellular hypertrophy were observed in mid- and high-dose males. Thyroid diffuse follicular cell hypertrophy was observed in the high-dose males. In the high-dose females, an increased relative thyroid weight, and increased incidences of thyroid focal cystic follicular cell hyperplasia as well as diffuse follicular cell hypertrophy were seen. The body weight gain was decreased throughout the study for the mid- and high-dose males and high-dose females. In light of the systemic effects observed, dosing in males at 90 mg/kg/day was considered to be adequate for assessing the carcinogenic

potential of thiabendazole in the rat. The increase in TSH and decrease in T3 levels seen at 90 mg/kg/day and above were supportive of the interference with thyroid-pituitary homeostasis. Females appear to be less sensitive at 30 and 90 mg/kg/day because the incidence of tumors was lower compared to males. However, the same spectrum of effects was seen in both sexes including the same target organ effects and a progression from non-neoplastic to neoplastic changes. Based on the above weight-of-evidence, the **CARC concluded** that the thyroid tumors in male rats at 30 and 90 mg/kg/day and in female rats at 90 mg/kg/day were treatment-related.

The **Registrant** contended that the findings of the submitted mechanistic study demonstrated that thiabendazole causes thyroid tumors in rats by interference with thyroid-pituitary homeostasis. The **CARC** concluded that although the registrant demonstrated the reversibility of the effect on thyroid hormones, they did not demonstrate the reversibility of the effect on the liver and thyroid weights as well as histopathological changes or effect on the liver enzymes was not examined. The CARC, however, considered that the available evidence based on liver changes and thyroxine clearance data is sufficient to demonstrate that the thyroid tumors are likely due to increased metabolism of thyroid hormone in the liver. When considered together, the available information suggests that thiabendazole interferes with thyroid-pituitary homeostasis.

! In the mouse carcinogenicity study, administration of thiabendazole in the diet was not associated with a significant increase in thyroid tumors. There was an increase in the absolute liver weight of high-dose females and an increase in the relative liver weight in mid-dose females and high-dose males and females. There was an increase in the relative liver: brain weight ratio in high-dose males and females. Although, there was dose-related increase in mortality in both sexes at mid and high dose at 18 month, sufficient number of animals survived to assess the carcinogenic response. Therefore, the CARC concluded that the mouse study was adequate to assess the carcinogenic potential of thiabendazole.

2. Mutagenicity: Although the CARC identified a datagap for a gene mutation assay, the Committee concluded that additional mutagenicity testing is not required to characterize the genotoxic potential of thiabendazole and that the current unpublished and published database satisfy guideline requirements for genotoxicty. The acceptable studies submitted by the registrant indicate that thiabendazole is not clastogenic in cultured mammalian cells and does not cause unscheduled DNA synthesis in cultured rat hepatocytes. However, a published study demonstrated that thiophanate-methyl which is converted to a benzimidazole was positive for the induction of micronuclei, but not structural chromosomal aberrations in mouse bone marrow cells *in vivo*. This finding suggests that it may interfere with the

mitotic spindle rather than cause structural chromosomal damage and is, therefore, aneugenic. This is consistent with positive mouse micronuclei data for MBC (methyl-2-benzimidazole carbamate) and benomyl. Both of these compounds also cause mouse liver tumors and teratogenic effects. Since aneuploidy may be involved in carcinogenesis, these findings suggest a secondary effect on DNA for the induction of tumors by thiabendazole.

3. Mode of Action: A mechanistic study was conducted to determine whether thyroid tumors in rats were due to antithyroid activity. In Sprague-Dawley Crl:CD BR male rats, thiabendazole at 90 and 270 mg/kg/day for 13 weeks increased the liver and thyroid weights accompanied by histopathological changes and increased circulating levels of TSH and decreased levels of T3. During treatment period, statistically significant increases in thyroxine clearance were noted in high-dose animals only. Withdrawal of thiabendazole treatment caused a reversal of the levels of TSH and T3. However, reversal of the thyroid and liver weight increases or changes in histopathology was not examined. The thyroid mechanistic study also did not evaluate responses on UDP glucuronosyltransferase enzyme. However, the CARC concluded that changes in liver and thyroxine clearance data support that the thyroid tumors are likely due to increased metabolism in the liver. The Committee considered that although these enzyme measurements are desirable, they are not required. The CARC also agreed that the overall available information suggests an interference by thiabendazole with thyroid-pituitary homeostasis.

Consideration of the Use of the Non-linear Extrapolation Approach for Thiabendazole

When evaluating thiabendazole, the Committee considered the possibility of using the non-linear extrapolation approach for thyroid neoplasms and the factors (as stated in the Science Policy Guidance; EPA, 1988b) in making the determination of whether the neoplasms are due to thyroid-pituitary imbalance. These include increases in cellular growth *in vivo*; hormone changes; site of action; dose correlations; reversibility; lesion progression; structure-activity analysis and other studies. The Committee also gave consideration to the extent to which genotoxicity may account for the observed tumor effects, the dose-response and the occurrence of tumors in other tissues in addition to the thyroid.

Conclusions: As indicated above, based on the overall judgement of the 8 types of data evaluating evidence for thyroid activity, the CARC concluded that there are sufficient data to determine whether or not there is suggestive evidence that the

thyroid tumors in the rat associated with administration of thiabendazole may be due to a disruption in the thyroid-pituitary homeostasis. In addition, the following factors were also considered in evaluating the carcinogenic potential of thiabendazole: (1) the incidence of thyroid tumors in rats was statistically significantly increased above controls and above the range observed in available historical control range in males at 30 and 90 mg/kg/day and in females at 90 mg/kg/day; (2) thiabendazole did not demonstrate mutagenic potential in gene mutation, chromosomal aberration, or DNA damage/repair assays but it is an aneugen *in vivo* in both somatic and germ cells; (3) liver tumors were observed in mice treated with the pesticide benomyl, which along with thiabendazole belongs to the benzimidazole group of compounds. Benomyl is aneugenic. However, benomyl did not affect the thyroid or induce thyroid tumors in rats nor did thiabendazole cause increase in the liver tumors in mice. The weight-of-the-evidence provides sufficient support that thiabendazole alters thyroid hormone homeostasis in male rats resulting in hypothyroidism. Thiabendazole causes increased liver weight and hepatocellular hypertrophy presumably via induction of microsomal enzymes. Enhanced hepatic metabolism of the thyroid hormones causes rapid clearance of T4, decreased level of T3 and a sustained release of TSH which in turn causes thyroid hypertrophy, hyperplasia and neoplasia. Lower doses of thiabendazole which failed to cause rapid clearance of T4 and increased the release of TSH showed no evidence of tumor induction. Therefore, the dose response should assume nonlinearity. The weight-of -the evidence indicates that thiabendazole is neither a mutagen nor a clastogen although it has been shown in the published literature to be weakly aneugenic in somatic and germinal cells. This information alone is NOT supportive of a linear mode of action for thyroid tumors because it is unclear whether aneuploidy is the cause or the effect of tumorigenesis. Similar to other antithyroid agents in rodents thiabendazole acts indirectly by causing sustained elevations in serum TSH levels associated with the development of thyroid carcinogenesis as oppose to rodent thyroid carcinogens that are directly DNA reactive. **When considered together, the available information supports the non-linear mode of action by interference with thyroid-pituitary homeostasis.**

4. Potential Carcinogenic Effects of Thiabendazole in Children: Risks to infants and children from environmental exposure of chemicals may differ qualitatively or quantitatively from those of adults due to potential differences in physiological and metabolic factors, toxicokinetics, toxicodynamics, diet, behavioral pattern. As stated in the July 1999 Draft revisions to the EPA's cancer risk assessment guidelines, when information is developed to show a mode of carcinogenic action that is expected to be relevant to adults, an evaluation needs to be made as to whether this mode of action is relevant to children. When there is no information on children, a "cogent biological rationale needs to be developed

regarding whether the mode of action is applicable to children." In the case of thiabendazole, there are no direct animal data evaluating its neoplastic potential from pre- and post-natal exposure, so reliance is placed upon a biological argument.

Rodents and humans share a common physiology with regard to the thyroid-pituitary feedback system. Short-term perturbation of this system often leads to similar effects in both species resulting in increases and decreases in circulating thyroid and pituitary hormones. It is well established in rodents that disruption of thyroid-pituitary status with elevation of TSH levels is associated with thyroid tumors. In the absence of chemical-specific data, humans and rodents are presumed to be equally sensitive to thyroid cancer due to thyroid-pituitary disruption (EPA, 1998b). Thus, assuming that thiabendazole interferes with thyroid-pituitary feedback system in humans like it does in SD rats, projections can be made as to potential consequences in children, using what is understood about the key events for its postulated mode of action in rats. The oral perinatal and prenatal studies revealed no evidence of increased susceptibility of rat, rabbit or mouse fetuses to *in utero* exposure to **thiabendazole**.

Thyroid hormones are regulated within rather narrow ranges, with normal adult human serum values often being given as T4--4 to 11 ug/dL and T3--80 to 180 ng/dL. TSH levels extend over a broader range--0.4 to 8 ug/ml, due to the incorporation in recent years of more sensitive laboratory methods that have extended the normal range to lower values (Ingbar &Woeber, 1981; Surks et al., 1990). The upper bound on normal TSH has not changed, and it is one of the important considerations of antithyroid effects of chemicals. During development somewhat higher levels for each of the hormones are noted, with adult hormone values being reached beyond about 10 years of age (Nicholson and Pesce, 1992). Growth of the thyroid gland continues for the first 15 years of life, going from about 1 gram at birth to an adult size of about 17 grams (Fisher and Klein, 1981; Larsen, 1982). Early developmental inability to synthesize adequate thyroid hormone leads to altered physical and mental development (cretinism) (DiGeorge, 1992; Goldey et al., 1995) and is treatable. The control of normal thyroid growth during development is not totally known, although the increase in gland size may be independent of TSH stimulation (Logothetopoulus, 1963). Extended deviations in human thyroid hormone levels either above or below the normal range are associated with hyperthyroidism and hypothyroidism, respectively and are treated in the U.S. to restore balance. It is recognized that the human thyroid is susceptible to ionizing radiation, the only verified human thyroid carcinogen. Children are known to be more sensitive than adults to the carcinogenic effects of radiation (NRC, 1990). The nature and consequences of radiation have differences from thyroid disruption by inborn deficits or possible chemical influence that is not

mutagenic. The major effect of ionizing radiation on the thyroid is thought to be due to mutation. Antithyroid effects can also be induced at elevated radiation doses due to cytotoxicity of follicular cells with resulting reduction in thyroid hormone and elevation of TSH. Mutagenic chemicals, however, do not act totally like radiation: (a) X rays penetrate the body and target organs without having to be absorbed. Chemicals must be absorbed and distributed to target organs. (b) Unlike most organic chemicals, radioiodine is actively transported and concentrated in the thyroid gland, and it becomes incorporated into nascent thyroglobulin. (c) Given that the size of the thyroid gland is smaller in children than in adults, for a given blood level of radioiodine, the internal dose to the thyroid of a child is greater than that for an adult. (d) Radioiodine in the Chernobyl accident was picked up by cattle and incorporated into milk. Due to differences in milk consumption, the external dose presented to children was greater than to adults. (e) Single quanta of radiation result in a series of ionizations within biological material, each of which can react with DNA to induce mutations and affect the carcinogenic process. Chemicals are much less efficient: they frequently need to be metabolized to active intermediates, with each molecule interacting singly with DNA, usually by forming adducts which can be converted to mutations. (f) The spectrum of mutagenic effects vary with the source. Ionizing radiation often results in deletions and other structural chromosomal aberrations, while chemicals not uncommonly produce more gene mutations. (g) The thyroid of children is more sensitive to carcinogenic effects of external radiation on a per unit dose basis than in adults, especially for children less than 5 years of age. Sensitivity decreases with advancing age and seems to disappear in adulthood. It is estimated that, overall, children may be two or more times more sensitive to carcinogenic effects of external emitters than are adults (NRC, 1990).

The evidence supports the view that thiabendazole's mode of action will not be different for children. Thyroid cancer (not due to radiation) is a rare condition in the U.S., occurring with an incidence of about 0.004% per year (Greenspan & Strewler, 1997). The incidence is predominantly in persons over 30, and increases in older persons; in children the incidence is at the 1 per million rate. Mortality rates per 100,000 are above zero only for those older than 35 (Ries et al., 1999). Therefore, it does not appear that the young have any propensity for thyroid cancer from which one could infer some underlying cancer process that differs from adults (absent ionizing radiation treatment or incidents, discussed above). The basic elements of thyroid function and hormone homeostasis are the same in children and adults. The chemical disruption mode of action of thiabendazole in animals, to the extent that it is applicable to humans, appears equally applicable to human subpopulations. It is not expected to share the features of radiation. **In conclusion, children are not expected to be more susceptible to**

thiabendazole-induced thyroid effects than adults.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA *Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Committee classified thiabendazole as **"likely to be carcinogenic to humans"** by the oral route based on the following weight-of-the-evidence consideration:

1. Thyroid tumors were seen in both sexes of rat. A dose-response in tumor incidence and progressive development of lesions were evident.

2. The relevance of the observed tumors to human exposure cannot be discounted.

3. The weight-of-the-evidence indicates that thiabendazole may interfere with thyroid-pituitary homeostasis.

VII. SELECTION OF "POINT OF DEPARTURE" ENDPOINT

The following data were considered in the selection of endpoint:

Table. 7 Thyroid Changes in Rats

Dose (mg/kg/day)								
Studies/Parameters Examined	0	10	30	40	90	160	270	320
MALES								
14-Week Subchronic Study (MRID 42942802) Non-neoplastic changes: Follicular cell hypertrophy	0/10	0/10	- -	1/10	- -	2/10	- -	6/10

2-Year Chronic/Carcino-genicity Study (MRID 43593201)								
Non-neoplastic changes								
Diffuse follicular cell hypertrophy	0/100	0/50	1/50-	- -	4/50	- -	- -	- -
Thyroid Adenomas (%)b	1/97	1/46	5/47	- -	6/46	- -	- -	- -
Mechanistic study (MRID 43593202)								
Hormone Levels:								
T3 (ng/dL)	104	106	- -	- -	97	- -	92	- -
TSH (µU/mL)	73	65	- -	- -	128	- -	144	- -
FEMALES								
14-Week Subchronic Study (MRID 42942802)								
Non-neoplastic changes:								
Follicular cell hypertrophy	0/10	0/10	- -	3/10	- -	10/10	- -	10/10
2-Year Chronic/Carcino-genicity Study (MRID 43593201)								
Non-neoplastic changes								
Diffuse follicular cell hypertrophy	0/100	0/50	0/50	- -	2/50	- -	- -	- -
Focal cystic follicular cell hyperplasia	3/100	0/50	3/50	- -	6/50	- -	- -	- -
Thyroid Tumors (%)	4/82	0/36	1/43	- -	5/44	- -	- -	- -

a Hormone levels were measured in male rats only
b Combined adenoma/carcinoma of thyroid
- -= Data not available

Since thyroid tumors were noted at 30 and 90 mg/kg/day in the rat chronic/carcinogenicity study and a decrease (statistically non-significant) in T3 (5-10% at 90 mg/kg/day and 11-19% at 270 mg/kg/day), rapid clearancce of T4 (at 270 mg/kg/day) and an increase (statistically significant) in TSH (66-160% at 90 mg/kg/day

and 65-189% at 270 mg/kg/day) levels was observed in the rat mechanistic study, 10 mg/kg/day was chosen as the "Point of Departure".

VIII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee made the following recommendations:

- # For human cancer risk assessment, the MOE approach was recommended. The use of the MOE approach is supported by the weight-of-evidence which suggests that thiabendazole may interfere with thyroid-pituitary homeostasis and does not act via a DNA-reactive or mutagenic mode of action.

- # For risk assessment, 10 mg/kg/day was chosen as the "point of departure" based on the findings of thyroid tumors at 30 mg/kg/day as well as decrease in T3 and increase in TSH at 90 mg/kg/day in males.

- # No separate risk assessment for thyroid tumors is needed for children since they are not expected to be more susceptible than adults.

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Subject: Comments on the Thiabendazole mouse study

From: Esther Rinde, Ph.D. Original (s) 11/09/99

To: Sanju Diwan, Ph.D.

I agree with the overall assessment and the classification for Thiabendazole, but wish to have the following included in the record:

At the Thiabendazole CARC meeting, we called the mouse carcinogenicity study unacceptable because there were several study deficiencies (variable dosing and protocol, variable sacrifice timing for animals and increased mortality in all groups).

An Ad Hoc meeting was subsequently convened which addressed these deficiencies and determined that the dosing was adequate and the study was considered acceptable. A calculation of survival in the various groups at 15 and at 18 months was inserted into the document (reproduced below).

% SURVIVAL

	Control 1	**Control 2**	**Control 3**	**Low Dose**	**Mid Dose**	**High Dose**
15 Months Females (65 weeks)	**100%**	**96%**	**96%**	**96%**	**94%**	**50%**
15 Months	**86**	**98**	**98**	**96**	**74**	**70**

Males (65 weeks)

18 Months Females (78 weeks)	**92**	**88**	**80**	**90**	**66**	**26**
18 Months Males (78 weeks)	**78**	**72**	**70**	**72**	**56**	**40**

I wish to point out that survival in female mice was very close to the cut-off at both 15 & 18 months (based on the test guidelines, *survival in a mouse chronic/onco study should not fall below 50% in any group at 15 months, or 25% at 18 months).*

Although this study would be "acceptable" according to our test guidelines, I think that in terms of assessing the carcinogenic potential for Thiabendazole, the study was compromised by the high mortality, especially in female mice. In females at the high dose: there were ***only 25*** animals left at 15 months and ***only 13*** animals at 18 months (vs 40-50 in controls). This means that the statistical power of this study was markedly reduced and **a less than potent carcinogen could easily have been missed**. I refer also to our proposed Cancer Guidelines which state: "A lack of tumorigenic responses at exposure levels that cause significant impairment of animal survival may also not be acceptable as negative findings because of the reduced sensitivity of the study".